Turk J Pharm Sci 11(3), 295-306, 2014

Original article

Spectrophotometric Determination of *p*-Phenylenediamine in Hair Dyes

Latha Saranya CH[1], Gurupadayya BM[1]*, Kinnera K[2] and Thejaswini JC[1]

[1]JSS University, JSS College of Pharmacy, Department of Pharmaceutical Analysis, Mysore 570 015, Karnataka, INDIA
[2]Novartis Healthcare Pvt. Ltd., Hyderabad-500033, INDIA

Two simple, sensitive, rapid, robust and reproducible spectrophotometric methods were developed for the determination of *p*-phenylenediamine (PPD) in hair dyes. The analysis of PPD was performed using alkaline solution of Folin's reagent and ninhydrin reagent in methanol at 453nm and 431nm respectively. The methods were linear in the concentration range from 2-12 µg/mL for Folin's reagent and 0.1-0.6 µg/mL for ninhydrin reagent. The methods were validated with respect to system suitability, linearity, precision, limit of detection (LOD), limit of quantification (LOQ), accuracy (recovery), ruggedness, and robustness. The developed methods can be used for routine analysis of *p*-phenylenediamine in marketed products. The methods were validated in accordance with the current ICH guidelines. The precision results, expressed by intra-day and inter-day relative standard deviation values, are satisfactory (RSD<2.00%).

Key words: Folin's reagent, *p*-Phenylenediamine, Ninhydrin reagent, Spectrophotometric method

Saç Boyalarındaki *p*-Fenilendiamin'in Spektrofotometrik Tayini

Saç boyalarında *p*-fenilendiamin (PPD) tayini için basit, hassas, hızlı, güçlü ve tekrarlanabilir iki spektrofotometrik yöntem geliştirilmiştir. PPD analizi sırasıyla 453 ve 431nm'de metanol içinde Folin ve ninhidrin reaktifinin alkali çözeltileri kullanılarak gerçekleştirilmiştir. Yöntemler Folin reaktifi için 2-12 µg / mL ve ninhidrin reaktifi için ise 0.1-0.6 µg / mL arası bir konsantrasyon aralığında doğrusaldır. Yöntemler sistem uygunluğu, doğrusallık, kesinlik, algılama (LOD) sınırı, miktar sınırı (LOQ), doğruluk (yeniden kazanım), sağlamlık ve dayanıklılık açısından valide edilmiştir. Geliştirilen yöntemler *p*-fenilenediamin içeren ürünlerin rutin analizi için kullanılabilir. Yöntemler, mevcut ICH kılavuzlarına uygun olarak değerlendirilmiştir. Gün içi ve günler arası bağıl standart sapma değerleri ile ifade edilen hassas sonuçlar tatmin edici (RSD <% 2.00) bulunmuştur.

Anahtar kelimeler: Folin reaktifi, *p*-Fenilendiamin, Ninhidrin reaktifi, Spektrofotometrik yöntem

***Correspondence**: E-mail: bm_guru2004@yahoo.co.in; Tel: +91 9242886136

INTRODUCTION

p-Phenylenediamine (PPD) (Figure 1) is a monocyclic aryl amine compound; its chemical formula is $C_6H_8N_2$ and its molecular weight is 108.15 g. It is a white to light purple powder that oxidizes turning first red, then brown then finally black on exposure to air (2). It is primarily used as an ingredient of oxidative hair coloring products at a maximal concentration of 4.0%. In addition to hair dyes, PPD may also be found in fur or textile dyes, photographic developing agent and as an antioxidant in rubber compounds. Individuals may be occupationally exposed to PPD during its manufacture or use, and the exposure may occur through inhalation, skin and/or eye contact, and ingestion (3).

Short-term exposure to high levels of PPD (acute effects) may cause severe dermatitis, eye irritation and tearing, asthma, gastritis, renal failure, vertigo, tremors, convulsions and coma in humans. Eczematous contact dermatitis may result from long-term exposure (chronic effect) in humans (4-6).

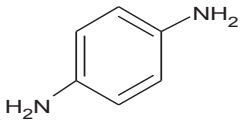

Figure 1. Chemical structure of *p*-phenylenediamine

Currently, PPD is present in more than 1000 hair dye formulations marketed all over the world (7). Epidemiologic studies demonstrated that workers in the textile dye and rubber industries, hair dye users and barbers incurred a high risk of bladder cancer, non-hodgkin's lymphoma, multiple myeloma and hematopoietic cancers (8). Carcinogens usually cause genomic damage to expose cells which may either undergo apoptosis or proliferation with genomic damage and potentially leading to transformation in cancerous cells (9).

Literature survey reveals some analytical methods are developed for the determination of PPD by HPLC (10-12), GC/MS (13,14), voltametric method (15), emission spectroscopy (16) and some spectrophotometric method are reported. These spectrophotometric methods have their relative merits but the methods are carried out with time consuming in diazotization followed by coupling with N-(1-naphthyl) ethylenediamine (17), involves oxidation of the compound converted in to salt measured colorimetrically (18), coupling of triclosan with reagent 2-aminonaphthalene-4,8-disulfonic acid with low level detection (19). The another method was based on the reaction of sodium nitrite with p-sulfanilic acid in an acidic medium to form diazonium ion, with which triclosan further formed an azo compound in an alkaline medium (20). Determination of triclosan in antiperspirant gels by first-order derivative spectrophotometry was also developed (21).

The present study was aimed to develop a simple, sensitive, rapid, reproducible, precise and accurate spectrophotometric method for the analysis of PPD using Folin's reagent and Ninhydrin reagent. The usage of these reagents is very common in laboratory and is very economic when compared to other reagents.

EXPERIMENTAL

Chemicals and Reagents

p-Phenylenediamine, 1,2-naphtho quinine-4-sulfonic acid sodium salt GR (Folin's Reagent) and sodium hydroxide was purchased from Loba Chemie Pvt. Ltd, Mumbai, India. Ninhydrin Extra pure AR was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Acetone was procured from Thomac Baker (Chemicals) Pvt. Ltd. Mumbai, India. All other chemicals used were of analytical grade (AR grade).

Instrumentation and Analytical Conditions

Shimadzu, Japan make UV-Visible double beam Spectrophotometer-1800 with quartz cuvette of 1 cm slit interval was employed for the present study. In addition, Shimadzu electronic balance, Japan and Millipore filtration assembly were used in this study.

The analysis was performed using alkaline solution of Folin's reagent using 0.1 N NaOH and 1% w/v ninhydrin in acetone.

Preparation of stock solutions
Preparation of standard stock solution

About 100 mg of pure sample of PPD was accurately weighed and dissolved in 100 mL of 0.1 N NaOH in a 100 mL standard flask to get a working standard concentration of about 1 mg/mL. From this solution, serial dilutions were made to obtain 100 µg/mL and 10 µg/mL.

Preparation of assay solution

0.833 g of marketed hair dye formulation containing 25 mg of PPD was weighed accurately and dissolved in 25 mL of 0.1 N NaOH solution to get a concentration of about 1 mg/mL. The solution is filtered using Whatmann filter paper and from these working samples of concentration falling in linearity range was prepared (5 µg/mL for Method-A and 0.25 µg/mL for Method-B) using 0.1 N NaOH solution.

Calibration curves
Method A

Standard solutions of PPD different aliquots 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 mL of 100 µg/mL were transferred into a series of 10 mL volumetric flasks, followed by the addition of 1.0 mL of Folin's reagent and 1 mL sodium hydroxide. The volume of this solution was diluted up to the mark with water and absorbance of each solution was measured at 453 nm against the reagent blank prepared in the same manner, without the analyte. The mechanism of action of PPD with Folin's reagent was shown in Figure 2.

Method B

Standard solutions of PPD different aliquots 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mL of 10 µg /mL were transferred into a series of 10 mL volumetric flasks, followed by the addition of 1.0 mL of Ninhydrin reagent. The volume of these solutions was diluted up to the mark with water and absorbance of each solution was measured at 431 nm against the reagent blank prepared in the same manner, without the analyte. The mechanism of action of PPD with ninhydrin reagent was shown in Figure 3.

Procedure for marketed formulation

Weighed amount of marketed hair dye formulation of 25 mg of PPD was transferred into a 25 mL volumetric flask. The content was shaken well in sonicator for 5 min with about 10 mL of 0.1N NaOH solution. The mixture was diluted to the mark of 25-mL with the same solution. It was filtered using Whatman No. 42 filter paper. First 10 mL portion of the filtrate was discarded and a subsequent portion was diluted to get a working concentration of 1mg/mL to analysis by taking 3 or 4 mL and following the procedure described earlier.

RESULTS AND DISCUSSION

Sodium 1,2-naphthoquinone-4-sulfonate (Folin's reagent) is a chemical reagent used to determine the amines and amino acids (22). The reagent produces a bright red color in alkaline solutions and is also fluorescent (23). The main advantage of this procedure of Folin's was its simplicity and a color was developed at room temperature in slightly alkaline solution. Folin's reagent have been used for the determination of many amino compounds and a large number of substances of pharmaceutical interest (24, 25). The mechanism of reaction between PPD and Folin's reagent are shown in the Figure. 2.

The ninhydrin reagent, one of the important reagent of detecting amino acids, both technically and historically, has been conventionally used to detect their microgram amounts. When amino acids with a free alpha amino group are treated with an excess of ninhydrin, they yield a purple colored product. Under appropriate conditions, the color intensity produced is proportional to the amino acid concentration. Ninhydrin is also used in amino acid analysis of proteins, most of the amino acids are hydrolyzed and reacted with ninhydrin except proline. The rest of the amino acids are then quantified colorimetrically after separation by chromatography. It has been extensively used in the determination of the compounds of pharmaceutical importance and in the kinetic studies (26,27). The chemical reaction of PPD and the reagent is depicted in Figure. 3.

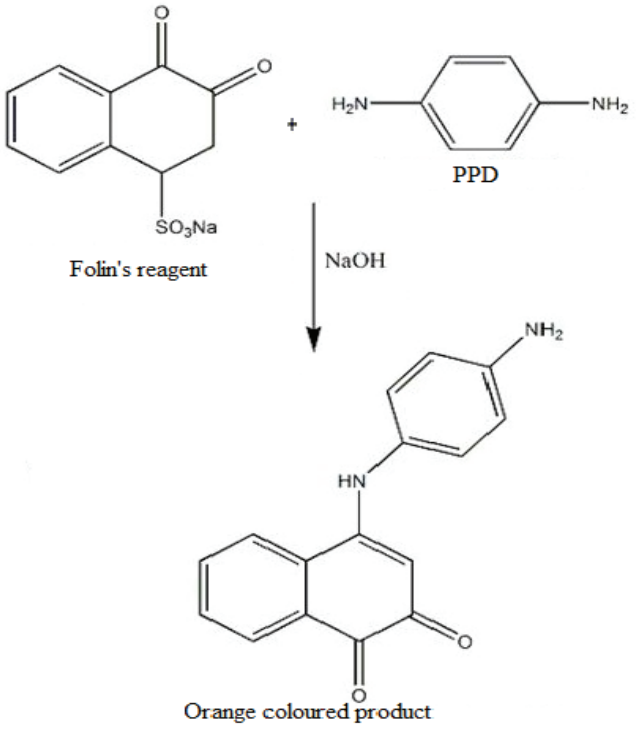

Figure 2. Mechanism of Folins reagent with PPD (Method A)

Method Development

Selection of wavelength

Standard stock solution of 100 µg/mL was prepared using 0.1 N NaOH solution as a solvent. From the stock solutions appropriate dilutions of PPD (10 µg/mL) were prepared and scanned over the range of 200 – 800 nm and the spectra was observed for development of suitable method for analysis. From the spectra of PPD wavelength were optimized at 453 nm and 431 nm when analysed with Folin's reagent and ninhydrin reagent respectively which were shown in Figures 4 and 5 respectively.

Method Validation

The method was validated for the parameters like system suitability, specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, ruggedness and robustness in accordance with International Conference of Harmonization (ICH) Guidelines.

System Suitability Testing

System suitability study was carried out by six replicate samples of the drug containing 5 µg/mL and 0.6 µg/mL of concentration for Method A and Method B respectively. System suitability of the methods was evaluated by analysing the absorbance and results are compiled in Table 1.

Molar absorptivity (L mol^{-1} cm^{-1}) = slope x 1000 x Molecular Weight

Sandell sensitivity (mg cm^{-2} per 0.001 absorbance unit) = $\frac{\text{least conc in µg/mL x 0.001}}{\text{absorbance of lest concentration}}$

Linearity

The calibration curve was established by plotting the absorbance of PPD versus concentration of PPD. Linear concentrations were found and described by the regression equations:

For method A: $y = 0.072x + 0.048$; $r^2 = 0.9919$,

For method B: $y = 1.5214x + 0.0267$, $r^2 = 0.9954$,

Where y is the absorbance PPD and x is the concentration in µg/mL, r^2 is the correlation coefficient. The Beer's law is obeyed in the concentration range of 2-12 µg/mL for Folins

and 0.1 – 0.6 µg/mL for Ninhydrin reagent. The results of the study are quite satisfactory and the results are compiled in Table 1.

Accuracy (Recovery study)

Accuracy of the method was studied by recovery experiments. The recovery was performed at three levels 80, 100 and 120% as per ICH guidelines. The present recovery experiments were performed at three level concentrations of PPD of 4, 5, 6 µg/mL by adding known amount pure drug of known concentration 5 µg/mL for method A and PPD of 0.2, 0.25, 0.3 µg/mL by adding known amount pure drug of known concentration 0.25 µg/mLfor method B. The results of the study are compiled in Tables 2 and 3 for Method A and B respectively and are quite satisfactory.

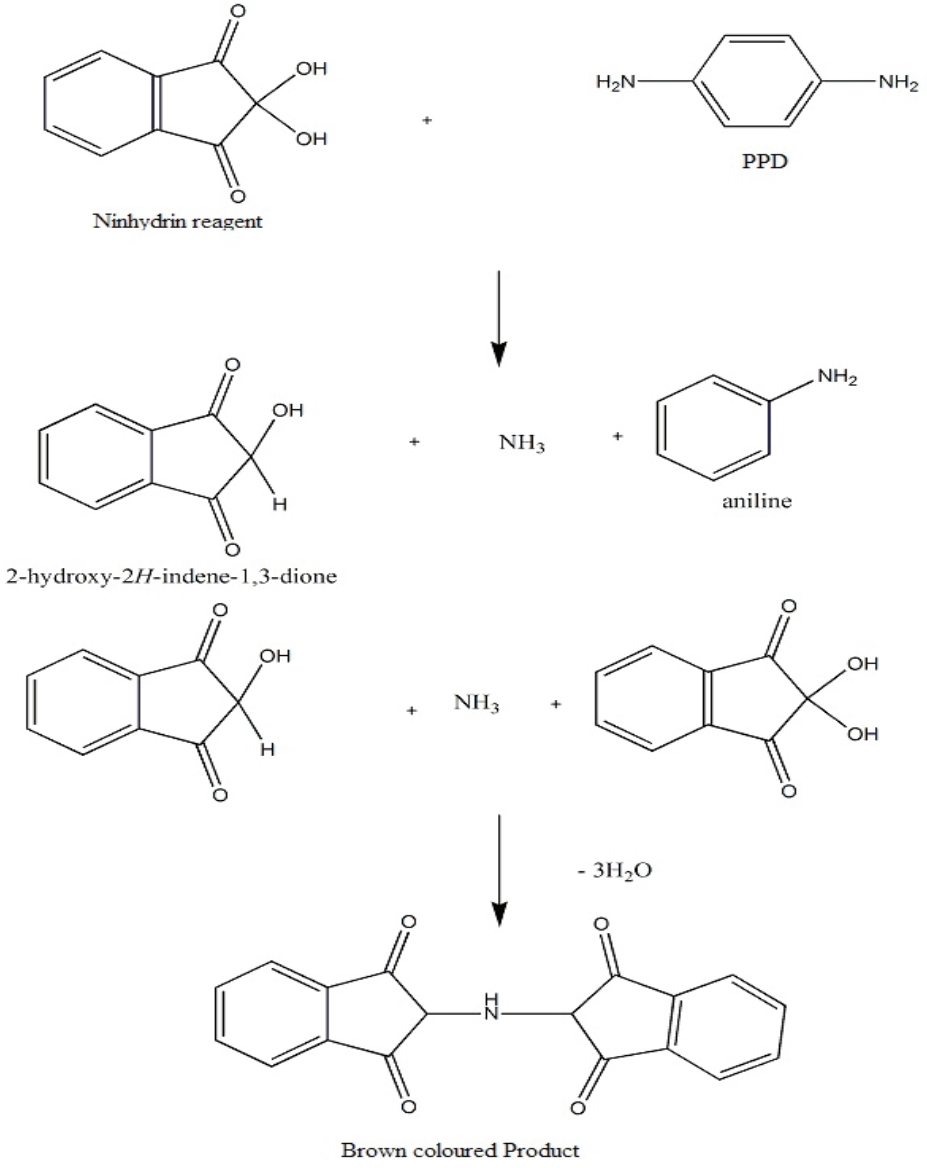

Figure 3. Mechanism of ninhydrin with PPD (Method B)

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were determined by using standard deviation of the response and

slope approach as defined in ICH guidelines. The limit of detection were found to be 0.0091 µg/mL and 0.0019 µg/mL for Method A and B respectively and limit of quantification were found to be 0.0277 µg/mL and 0.0059 µg/mL for Method A and B respectively.

$$LOD = 3 \frac{\text{SD of intercept}}{\text{slope}}$$

$$LOD = 10 \frac{\text{SD of intercept}}{\text{Slope}}$$

SD – Standard Deviation

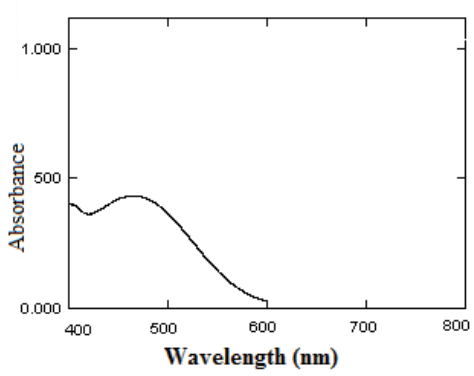

Figure 4. Absorption spectra of PPD with Folin's reagent

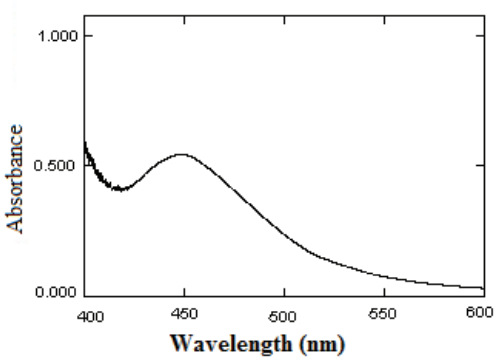

Figure 5. Absorption spectra of PPD with ninhydrin reagent

Precision

Precision of the methods were evaluated in terms of intra-day and inter-day precision. 8 µg/mL (method A) and 0.3 µg/mL (method B) of PPD were analysed in six replicates on the same day (intra-day precision) and in three consecutive days (inter-day precision). The absorbance based intra-day % RSD value was 0.1227 and 0.173 for method A and B respectively. The inter-day precision showed % RSD values of 0.070 and 0.123 for method A and B respectively. The results of the study are compiled in (Tables 4, 5) and are quite satisfactory.

Ruggedness

Method ruggedness was checked by varying the lot number and manufacturers of reagents, solvents (0.1 N NaOH solution, methanol, and deionised water and different absorbance ranges. The effect of changes was observed on absorbance, λmax, linearity, regression coefficient.

Robustness

The experiments were performed by slightly varying the experimental conditions like the proportions of the solvent (+/- 2% on total proportion), concentration of solvent, temperature of the samples (30 +/- 5^0C) and wavelength (+/- 2 nm) of detection. The effect of changes was observed on absorbance, λmax, linearity, regression coefficient.

The results were in agreement with the labelled amounts. For comparison, HPLC method (28) was used for parallel comparison and results are shown in Table 7. The proposed method does not require any heating/extraction or use no expensive chemicals. The methods are highly sensitive and economic compared with HPLC method.

CONCLUSION

The Spectrophotometric determination of PPD in hair dyes was performed successfully. The developed methods were found to be economic, novel, simple, sensitive, accurate, precise and reproducible; it can be used for routine analysis of PPD and marketed hair dye products.

Table 1. Optical parameters for method A and B

Parameters determined	Obtained values	
	Method A	**Method B**
λ_{max}	453 nm	431 nm
Linearity (µg/mL)	2 - 12	0.1 - 0.6
Slope ± SEM	0.0721± 0.003	1.5227 ± 0.01
Intercept ± SEM	0.0487 ± 0.004	0.0276 ± 0.003
Regression coefficient	0.9919	0.9954
Wavelength	453 nm	431 nm
LOD (µg/mL)	0.0091	0.0019
LOQ (µg/mL)	0.0277	0.0059
Molar absorptivity (L mol^{-1} cm^{-1})	7.8×10^3	1.6×10^5
Sandell sensitivity (mg cm^{-2} per 0.001 absorbance unit)	0.009 A.U.	0.0004878 A.U.

Table 2. Accuracy (Recovery studies) for Method A

S. No	Amount of drug taken in µg/mL		Total amount of drug in µg/mL	Total amount of drug found µg/mL	% Recovery	Average recovery in %	% RSD
	marketed sample	Pure added					
1	04	05	09	8.876	98.625	98.77 ±0.1381 (SEM)	0.312
				8.914	99.05		
				8.841	98.23		
				8.876	98.625		
				8.914	99.05		
				8.915	99.05		
				9.787	97.87		
				9.95	99.5		
2	05	05	10	10.049	100.49	102.06 ± 0.9414 (SEM)	0.867
				9.852	98.52		
				9.95	99.5		
				9.852	98.52		
3	06	05	11	10.948	99.53	99.423 ± 0.3498 (SEM)	0.321
				10.918	99.25		
				11.008	100.0		
				10.948	99.53		
				10.888	98.98		
				10.918	99.25		

SEM – Standard Error of Mean

Table 3. Accuracy (Recovery studies) for Method B

S. No	Amount of drug taken (µg/mL)		Total amount of drug in µg/mL	Total amount of drug found % / taken%	% Recovery	Average recovery in %	% RSD
	Formulation	Pure added					
1	0.2	0.25	0.45	0.445	99.01	98.46 ± 0.155 (SEM)	0.457
				0.442	98.35		
				0.442	98.35		
				0.439	97.7		
				0.442	98.35		
				0.445	99.01		
2	0.25	0.25	0.5	9.963	99.63	99.28 ± 0.141 (SEM)	0.394
				9.910	99.10		
				9.963	99.63		
				9.858	98.58		
				9.963	99.63		
				9.910	99.10		
3	0.3	0.25	0.55	0.547	99.58	99.72 ± 0.137 (SEM)	0.332
				0.550	100.00		
				0.545	99.16		
				0.547	99.58		
				0.550	100.00		
				0.5500	100.00		

SEM – Standard Error of Mean

Table 4. Method Precision Method A and B

Method	Concentration (µg/mL)	Intra day			Inter day		
		Mean ± SEM	SD	% RSD	Mean ± SEM	SD	% RSD
A	8	0.621 ± 0.01**	0.001213	0.1227	0.614 ± 0.14*	0.000471	0.07
B	0.3	1.009± 0.03**	0.000816	0.173	0.991 ± 0.18*	0.000574	0.123

Average values were expressed in Mean + SEM. **p< 0.01, * p< 0.05, ns: p> 0.05 when compared with only reagent. Statistical analysis were performed by Two way ANOVA followed by Dunnett test.

Table 5. System precision

Samples	Absorbance	
	Method A	**Method B**
1	0.220	0.636
2	0.358	0.868
3	0.503	1.011
4	0.627	1.214
5	0.771	1.416
6	0.887	-
Mean	0.561 ± 0.03**	1.029 ± 0.09**
S.D.	0.229	0.302
% RSD	0.408	0.293

Average values were expressed in Mean + SEM. **p< 0.01, * p< 0.05, ns: p> 0.05 when compared with only reagent. Statistical analysis were performed by Two way ANOVA followed by Dunnett test.

Table 6. Ruggedness and robustness

S.No	Parameter		Variation	Inference
1	Solvent		0.1N NaOH	RSD ≤ 2%
			Methanol	Absorbance spectra is not clear
			De ionized water	λ_{max} is not satisfactory
2	Wavelength	Folin's reagent	451 nm	RSD ≤ 2%
			455 nm	RSD ≤ 2%
		Ninhydrin reagent	429 nm	RSD ≤ 2%
			433 nm	RSD ≤ 2%
3	Different analyst			RSD ≤ 2%
4	Different day			RSD ≤ 2%
5	pH		± 0.2 - ± 0.4	Stability was not in acceptable range (turbidity formation)
6	Vortex time		4.5-5.5 min	No changes were observed in absorption spectra

Table 7. Comparison of reported HPLC methods with proposed method

Parameters	Proposed method		Reference method
	Method A	**Method B**	
Accuracy (Recovery studies)	98.77 -102.06	98.46 -99.72	91.32-96.45
Linearity (µg/mL)	2 - 12	0.1 - 0.6	0-900
Wavelength (nm)	453	431	235
Detection limit (µg/mL)	0.0091	0.0019	0.44
Regression coefficient	0.9919	0.9954	0.9976

ACKNOWLEDGEMENT

The authors are thankful to VGST-SPiCE, Science and Technology, Karnataka, India for financial support to carry out the project work.

REFERENCES

1. Environmental Protection Agency Health and Environmental Effects Profile for Phenylenediamines. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Office of Research and Development. Cincinnati, OH, USA, 1985.
2. Department of Health and Human Services Hazardous Substances Data Bank (HSDB, online database). National Toxicology Information Program, National Library of Medicine. Bethesda, MD, USA, 1993.
3. Scientific Committee on Consumer Products (SCCP) Opinion on P-phenylenediamine, Public Health and Risk Assessment, 9th plenary meeting, Brussels, Belgium 10(10), 2006.
4. Kind F, Scherer K, Bircher AJ, Contact dermatitis to para-phenylenediamine in hair dye following sensitization to black henna tattoos-an ongoing problem, J Dtsch Dermatol Ges 10(8), 572-578, 2012.
5. Jacob SE, Zapolanski T, Chayavichitsilp P, Sensitivity to para-phenylenediamine and intolerance to hydrochlorothiazide, J Dermatitis 19(6), 44 - 45, 2008.
6. Lepoittevin P, LeCoz CJ, Paraphenylene-diamine. In: Dictionary of Contact Allergens, Ed (1): Springer, pp. 194-196, Berlin, Germany, 2007.
7. Stanley LA, Skare JA, Doyle E, Powrie R, D'Angelo D, Elcombe CR, Lack of evidences for metabolism of p-phenylenediamine by human hepatic cytochrome P450 enzymes, J Toxicol 210(2-3), 147-157, 2005.
8. Thun MJ, Altekruse SF, Namboodiri MM, Calle EE, Myers DG, Personal of hair dyes and the risk of blood cancer: results of a meta-analysis, Public Health Rep 120(1), 31-38, 2005.
9. Steller H, Mechanisms and genes of cellular suicide Science. Science 3(10), 1445-1449, 1995.
10. Al-Suwaidi A, Ahmed H. Determination of para-phenylenediamine (PPD) in henna in United Arab Emirates. Int J Environ Res Public Health 7(4), 1681-1693, 2010.
11. Yoshiaki Ikarashi, Masa-aki Kaniwa, Determination of p-phenylenediamine and related antioxidants in rubber boots by high performance liquid chromatography. Development of an analytical method for N-(1-Methylheptyl)-N′-phenyl-p-phenylenediamine. J Health Science 46(6), 467 - 473, 2000.
12. Ursula Vincent, Guy Bordin, and Adela R. Rodrigue, Validation of an analytical procedure for the determination of oxidative hair dyes in cosmetic formulations, J Cosmet Sci 53, 43-58, 2002.
13. Maria Luisa Di Gioiaa, Antonella Leggioa, Adolfo Le Peraa, Angelo Liguoria, Determination by gas chromatography/mass spectrometry of p-phenylenediamine in hair dyes after conversion to an imine derivative, J Chromatogr A 1066 (1–2), 143–148, 2005.
14. Stambouli A, Bellimam MA, El Karni N, Bouayoun T, El Bouri A, Optimization of an analytical method for detecting paraphenylenediamine (PPD) by GC/MS-iontrap in biological liquids, Forensic Sci Int 146, S87-S92, 2004.
15. Inzhang Gao; Xiaodong Chen Jie Ren; Wu Yang, Determination of p-phenylenediamine by perturbation of a non-equilibrium stationary state in the B-Z reaction, Journal of the Brazilian Chemical Society, J Braz Chem Soc 22 (4), 648-651, 2011.
16. Kessarin Ngamdee Surangkhana Martwiset Thawatchai Tuntulani Wittaya Ngeontae, Selective fluorescence sensors for p-phenylenediamine using formyl boronate ester with an assistance of micelles, Sensors & Actuators: B. Chemical, 173, 682-691, 2012.
17. Nitin S Jadhav, Muthu Lakshmi M, Meena S, Duraivel S, Validated visible spectrophotometric estimation of para-phenylenediamine, a carcinogenic ingredient in henna hair dyes, Int J Pharm Technol 2(4), 900-906, 2010.
18. Hilton CL, Spectrophotometric determination of flexzone 3C and other p-phenylenediamine derivatives, Anal Chem 32 (12), 1554–1557, 1960.
19. Olexander IK, Natalia IS, Visual spectroscopy detection of triclosan, Chemical Papers 64(4), 523-527, 2010.
20. Huihui Lu, Hongbing Ma, Guanhong Tao, Spectrophotometric determination of triclosan in personal care products, Spectrochimica Acta Part A: Molecular and Biomolecular spectroscopy, 73(5), 4, 2009.
21. Du L, Li M, Jin Y, Determination of triclosan in antiperspirant gels by first-order derivative

spectrophotometry, Pharmazie 66(10, 740-743, 2011.
22. Saurina J, Hernández-Cassou S, Determination of amino acids by ion-pair liquid chromatography with post-column derivatization using 1,2-naphthoquinone-4-sulfonate, J Chromatogr A 676(2), 311-319, 1994.
23. Kobayashi Y, Kubo H, Kinoshita T, Fluorometric determination of guanidino compounds by new postcolumn derivatization system using reversed-phase ion-pair high-performance liquid chromatography, Anal Biochem 160, 392-398, 1987.
24. Navya Sloka S, Gurupadayya BM, Aswani Kumar C, Spectrophotometric determination of mesalamine by PDAC and NQS reagents in bulk and tablet dosage form, J Pharm Res 4(1), 39-41, 2011.
25. Aswani Kumar C, Gurupadayya BM, Navya Sloka S, Chandan RS, Thejaswini JC, Colorimetric determination of cefadroxil and ceftriazone in pharmaceutical dosage forms, Trop J Pharm Res 10(1), 81-88, 2011.
26. Nafisur Rahman, Syed Najmul Hejaz Azmi, Spectrophotometric method for the determination of amlodipine besylate with ninhydrin in drug formulations, Il Farmaco 56(10), 731–735, 2001.
27. Nafisur Rahman, Mohammad Kashif, Application of ninhydrin to spectrophotometric determination of famotidine in drug formulations, Il Farmaco, 58(10), 1045–1050, 2003.
28. Ursula Vincent, Guy Bordin, and Adelar R Odrigu, Validation of an analytical procedure for the determination of oxidative hair dyes in cosmetic formulations, J Cosmet Sci 53 (01), 43-58, 2002.

Received: 07.11.2013
Accepted: 13.02.2014